

**REMARKS**

Reconsideration is requested.

Claims 1-12 and 14-19 have been canceled, without prejudice. Claims 13 and 20 are pending.

Support for the amendments can be found, for example, in the specification as filed at page 2, lines 25-37 to page 3, lines 1-9, page 4, lines 32-35 and page 5, lines 27-34. No new matter has been added.

To the extent not obviated by the above, the Section 102 rejections of claims 13, 14 and 16, over Harding (J. Immunology, 151:3988-3998, 1993) and claims 13 and 15 over Amigorena (Nature 369:113-120, 1994) are traversed. Reconsideration and withdrawal of the rejection are requested in view of the following distinguishing comments.

The instantly disclosed and claimed method of isolating exosomal vesicles is distinct from the method of isolating lysosomal vesicles taught in the prior art.

The Examiner asserts that

“there is no evidence that the vesicles themselves are any different” (see page 3 of the Office Action dated October 19, 2004)

The Examiner is understood to believe that the last paragraph of page 7 of the instant specification equates the “exosomes” described on pages 2-3 with the “vesicles” of the claims and discloses that the “vesicles” are morphologically similar to MICs.

Assuming the applicant have understood the Examiner’s position correctly, the applicant respectfully disagrees with the Examiner’s interpretation of the specification as the last paragraph of page 7 of the specification states the following:

"The vesicles were morphologically similar to those [vesicles] present in MIICs [...]"

Accordingly, the specification teaches that MIICs are different from vesicles, but may contain vesicles which are similar thereto. This paragraph also states that

"The 70,000 x g pellets were composed of a homogeneous population of vesicles labelled for MHC class II (Figure 2 B)"

Vesicles distinct from the lysosomes/MIICs of the prior art may thus be obtained with a method according to the invention comprising:

subjecting cell culture media obtained from a B lymphocytes culture, said B lymphocytes containing MHC Class II protein, to differential centrifugation; and  
recovering a 70,000 g pellet from said differential centrifugation, said pellet containing said vesicle.

The applicant, who is also a co-author of the cited Harding et al., submits that there are at least the following three main physical differences between lysosomes of the cited prior art and antigen presenting vesicles of the claimed invention:

- (1) the vesicles, whose size ranges from 60 to 80 nm, are about 1/10 smaller than lysosomes (which are 0.5  $\mu$ m large);
- (2) the vesicles of the invention are morphologically distinct from lysosomes; and
- (3) when collected from the culture media of cells through density centrifugation, the vesicles are found at a density different from the density of the lysosomes.

The applicant further submits that vesicles of the claimed invention are secreted by cells whereas lysosomes do not leave the cells (lysosomes do not result from a spontaneous vesicle exocytosis). Barral et al. ("*Exosomes: Specific Intercellular Nano-Shuttles?*", Ana Maria Barral and Matthias G. von Herrath; Current immunology

Reviews, 2005, 1, 1-6), copy attached and listed on the attached PTO 1449 Form,  
further indicates that

"The process of exosome release is, similarly to virus budding and milk-fat globule secretion, a reverse budding event, where exosomes will contain cytosol inside, exposing the extracellular (or luminal when coming from the endosomal pathway) domain of receptors." (see sentence spanning left and right columns, page 3 and also Figure 1).

According to Barral et al., exosomes can also

"be identified both by morphological and biochemical criteria. [...] The presence of lysosomal proteins in exosomes is partial and dependent on their cellular origin. Thus, exosomes in general do not contain lysosomal proteases or other soluble residents. For instance B-cell exosomes do not contain the invariant chain CD74 and LAMP-2 [...] Lipid composition of exosomes is similar to that of lipid rafts in that it is enriched in cholesterol and sphingomyelin. Other raft markers have been detected in exosomes, such as GM1, GM3, flotillin and the src protein kinase Lyn. [...] In Table 1, a summary of the main protein families encountered in exosomes is shown." (see right column, page 3 and left column, page 4).

The attached reference and attached is submitted to demonstrate that the vesicles of the claimed invention are physically distinct from lysosomes of the cited art, Claim 13 is submitted to be patentable over the cited art and withdrawal of the Section 102 rejections of claim 13 is requested.

For completeness, the applicant urges the Examiner to appreciate that Harding et al. and Amigorena et al. do not describe or suggest the ability of antigen presenting vesicles according to the claimed invention to stimulate immune responses when conveniently purified from the extracellular fluid.

Geuze  
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Amendment

The Section 112, second paragraph, rejection of claim 20 is obviated by the above amendments. Withdrawal of the rejection is requested.

The Section 112, second paragraph, rejection of claims 18-19 is moot.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

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